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DATE: Monday, September 18, 2006

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Γ	L8	(process\$5 or degrad\$5 or synthes\$5) same L6	11
Γ	L7	carbohydrate same L6	0
<u> </u>	L6	(mutant or variant or modif\$5) same L5	35
Ļ	L5	(gene or sequence or polynucleotide) same L4	379
<u>; </u>	L4	(glycosyl adj hydrolase)	626
Γ	L3	beta and L2	28
Γ	L2	glycosidase and L1	38
_	L1	davis.in.	43791

END OF SEARCH HISTORY

=> index bioscience medicine

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ... 'ENTERED AT 12:26:20 ON 18 SEP 2006

71 FILES IN THE FILE LIST IN STNINDEX

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=> S (glycosyl (w) hydrolase)
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- 85 FILE BIOENG
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- 177 FILE BIOTECHNO
- 98 FILE CABA
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- 14 FILE CEABA-VTB
- 4 FILE CONFSCI
- 1 FILE CROPU
- 4 FILE DDFB
- 1 FILE DDFU
- 848 FILE DGENE
- 19 FILE DISSABS
- 4 FILE DRUGB
- 1 FILE DRUGU
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- 10 FILE FROSTI
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 - 87 FILE TOXCENTER269 FILE USPATFULL
 - 51 FILE USPAT2
 - I FILE VETB
 - 1 FILE WATER
 - 39 FILE WPIDS
 - 39 FILE WPINDEX
 - I FILE NLDB

40 FILES HAVE ONE OR MORE ANSWERS, 71 FILES SEARCHED IN STNINDEX

L2 QUE (GLYCOSYL (W) HYDROLASE)

-> d rank

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- F2 848 DGENE
- F3 458 CAPLUS
- F4 417 BIOSIS
- F5 416 SCISEARCH
- F6 339 ESBIOBASE
- F7 294 MEDLINE F8 269 USPATFULL
- F9 258 LIFESCI
- FIO 247 EMBASE

177 BIOTECHNO F12 122 PASCAL F13 114 BIOTECHABS 114 BIOTECHDS F14 105 FSTA F15 F16 98 CABA F17 87 TOXCENTER F18 85 BIOENG F19 70 AGRICOLA F20 51 USPAT2 39 WPIDS F21 F22 39 WPINDEX F23 35 JICST-EPLUS F24 32 IFIPAT F25 19 DISSABS F26 14 CEABA-VTB 11 AQUASCI F27 F28 10 FROSTI F29 4 CONFSCI F30 4 DDFB 4 DRUGB F31 F32 4 EMBAL F33 4 OCEAN F34 I AQUALINE F35 I CROPU F36 I DDFU F37 1 DRUGU F38 1 VETB F39 1 WATER F40 1 NLDB

F11

=> file f3-f15

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=> s L2

L3 3216 L2

=> S (gene or sequence or polynucleotide)(s)L3

9 FILES SEARCHED...

1563 (GENE OR SEQUENCE OR POLYNUCLEOTIDE)(S) L3

=> S (mutant or variant or modif?)(s) L4

L5 105 (MUTANT OR VARIANT OR MODIF?)(S) L4

=> S (process? or degrad? or synthes?)(s)L5

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38 (PROCESS? OR DEGRAD? OR SYNTHES?)(S) L5

=> S carbohydrat? (s)L6

6 CARBOHYDRAT? (S) L6 1.7

=> dup rem 17

PROCESSING COMPLETED FOR L7

6 DUP REM L7 (0 DUPLICATES REMOVED)

=> d ibib abs L8 1-6

L8 ANSWER I OF 6 USPATFULL on STN

2006:34176 USPATFULL << LOGINID::20060918>> ACCESSION NUMBER:

Novel full length cDNA TITLE:

INVENTOR(S): Isogai, Takao, Ibaraki, JAPAN

Sugiyama, Tomoyasu, Tokyo, JAPAN

Otsuki, Tetsuji, Kisarazu-shi, JAPAN

Wakamatsu, Ai, Kisarazu-shi, JAPAN

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Masuho, Yasuhiko, Tokyo, JAPAN

PATENT ASSIGNEE(S): RESEARCH ASSOCIATION FOR BIOTECHNOLOGY (non-U.S. corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 2006029945 A1 20060209

APPLICATION INFO.: US 2005-72512 A1 20050307 (11)

RELATED APPLN. INFO.: Division of Ser. No. US 2002-104047, filed on 25 Mar

2002, GRANTED, Pat. No. US 6943241

NUMBER DATE

PRIORITY INFORMATION: JP 2001-379298 20011105

US 2002-350978P 20020125 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: FOLEY AND LARDNER LLP, SUITE 500, 3000 K STREET NW,

WASHINGTON, DC, 20007, US

NUMBER OF CLAIMS: 5

EXEMPLARY CLAIM:

NUMBER OF DRAWINGS: 1 Drawing Page(s)

LINE COUNT: 12974

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel full-length cDNAs are provided. 1970 cDNA derived from human have been isolated. The full-length nucleotide sequences of the cDNA and amino acid sequences encoded by the nucleotide sequences have been determined. Because the cDNA of the present invention are full-length and contain the translation start site, they provide information useful for analyzing the functions of the polypeptide.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 2 OF 6 USPATFULL on STN

ACCESSION NUMBER: 2005:131264 USPATFULL << LOGINID::20060918>>

TITLE: Secreted and transmembrane polypeptides and nucleic

acids encoding the same

Ashkenazi, Avi J., San Mateo, CA, UNITED STATES INVENTOR(S):

Baker, Kevin P., Damestown, MD, UNITED STATES

Botstein, David, Belmont, CA, UNITED STATES

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Wood, William I., Hillsborough, CA, UNITED STATES

Zhang, Zemin, Foster City, CA, UNITED STATES

PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, UNITED STATES (U.S. corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 2005112725 A1 20050526

APPLICATION INFO.: US 2004-978255 A1 20041029 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-989862, filed on 19

Nov 2001, PENDING Continuation of Ser. No. US

2001-941992, filed on 28 Aug 2001, PENDING Continuation

of Ser. No. WO 2000-US8439, filed on 30 Mar 2000,

PENDING Continuation-in-part of Ser. No. US 380137, ABANDONED A 371 of International Ser. No. WO

1999-US12252, filed on 2 Jun 1999

NUMBER DATE

PRIORITY INFORMATION: US 1999-141037P 19990623 (60)

US 1998-88810P 19980610 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: HELLER EHRMAN WHITE & MCAULIFFE LLP, 275 MIDDLEFIELD

ROAD, MENLO PARK, CO, 94025-3506, US

NUMBER OF CLAIMS: 24

EXEMPLARY CLAIM: 1-118

NUMBER OF DRAWINGS: 330 Drawing Page(s)

LINE COUNT: 38226

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to novel polypeptides and to nucleic

acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

```
L8 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-21532 BIOTECHDS <<LOGINID::20060918>>
             Novel modified polypeptide with carbohydrate processing
TITLE:
          enzymatic activity, including substitution of amino acid
          residue forming catalytic nucleophile of active site, by less
          nucleophilic residue, useful for hydrolyzing beta glycoside;
            involving vector-mediated gene transfer and expression in
            host cell for therapy
                DAVIS B G
AUTHOR:
PATENT ASSIGNEE: ISIS INNOVATION LTD
                 WO 2005059126 30 Jun 2005
PATENT INFO:
APPLICATION INFO: WO 2004-GB5266 15 Dec 2004
PRIORITY INFO: GB 2003-29011 15 Dec 2003; GB 2003-29011 15 Dec 2003
DOCUMENT TYPE: Patent
LANGUAGE:
                 English
OTHER SOURCE: WPI: 2005-497551 [50]
AN 2005-21532 BIOTECHDS << LOGINID::20060918>>
AB DERWENT ABSTRACT:
   NOVELTY - A ***modified*** polypeptide (P1) having
    ***carbohydrate*** ***processing*** enzymatic activity, and
   comprising a ***modification*** that includes substitution of the
   amino acid residue forming the catalytic nucleophile of an active site,
   by a less nucleophilic amino acid residue, which retains some
   nucleophilic activity, is new.
      DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
   following: (1) a ***polynucleotide*** (P2) encoding (P1); (2)
   expression vector (V1) comprising (P2); and (3) host cell transformed
   with (V1).
      WIDER DISCLOSURE - The following are disclosed: (1) microarray
   comprising (P2); and (2) non-human animals comprising (P2).
      BIOTECHNOLOGY - Preferred Polypeptide: (P1) comprises amino acid
    ***sequence*** chosen from (a) a fully defined 489 amino acid (SEQ ID
   No. 2) ***sequence*** given in the specification, comprising a
   substitution of an amino acid equivalent to an amino acid residue Glu at
   position 387, by a less nucleophilic residue, (b) amino acid
   ***sequence*** of family I ***glycosyl*** ***hydrolase*** comprises a substitution of an amino acid equivalent to an amino acid
                                                   ***hydrolase***, that
   residue Glu at position 387 of SEQ ID No. 2, by a less nucleophilic
   residue, and (c) a ***variant*** of amino acid ***sequence*** of
   (a) or (b) having ***carbohydrate*** ***processing*** enzymatic
   activity and comprising a substitution at a position equivalent to Glu at
   position 387 of SEO ID No. 2 by a less nucleophilic residue, where the
   less nucleophilic residue retains nucleophilic activity. The less
   nucleophilic amino acid residue is chosen from tyrosine, asparagine,
   cysteine, glutamine and arginine. (P1) has glycosyl synthase,
    ***glycosyl*** ***hydrolase*** and/or transglycosylase activity.
   The family 1 ***glycosyl*** ***hydrolase*** is Sulfolobus
   solfataricus beta-glycosidase. (P1) further comprises one or more
   mutations chosen to broaden the substrate specificity of the polypeptide
   compared to a polypeptide not so ***modified***, where the mutations
   are chosen from (a) at least one of W433, E432 and M439 of SEQ ID No. 2,
   (b) at least one amino acid residue equivalent to W433, E432 and M439 of
   SEQ ID No. 2 in the amino acid ***sequence*** of family 1
    ***glycosyl*** ***hydrolase***, and (c) at least one amino acid
   mutation at a position equivalent to W433, E432 and M439 of SEQ ID No. 2
   in a ***variant*** of (a) or (b) having ***carbohydrate***
     ***processing*** enzymatic activity. (P1) comprises (a) SEQ ID No. 2
   having one or more of amino acid residues such as Trp, Glu and Met at
   position 433, 432 and 439, substituted by cysteine, valine and alanine;
   (b) the amino acid ***sequence*** of family 1 ***glycosyl***
```

hydrolase, having at least one amino acid residue equivalent to

Trp, Glu and Met at position 433, 432 and 439 of SEQ ID No. 2, substituted by cysteine, valine and alanine; or (c) ***variant*** of (a) or (b) having at least one amino acid residue equivalent to Trp, Glu and Met at position 433, 432 and 439 of SEQ ID No. 2, substituted by cysteine, valine and alanine.

USE - (P1) is useful for hydrolyzing, ***synthesizing*** or transglycosylating beta glycoside, which involves contacting a glycoside substrate with (P1), where the glycoside substrate is chosen from glucoside, galactoside, fucoside, xyloside, mannoside and glucuronide. (P1) is contacted with a sample containing at least two different glycosides (claimed). (P1) is useful for generating glycoproteins, therapeutic molecules, and antibiotics in particular macrolide antibiotics. (P1) is useful in food industry to achieve depulping, in detergents, and in therapeutical applications. (P1) is useful in developing glycoconjugates for use in lectin enzyme activated prodrug system.

ADVANTAGE - (P1) has glycosyl synthase, ***glycosyl***

hydrolase and/or transglycosylase activity (claimed). (P1) is
compatible at high temperature and in organic solvents, and forms
glycosidic linkages between two monosaccharides, without the need for
protection or activation steps, thus acting as super beta-catalyst. (I)
can be produced in large-scale and is purified by HPLC techniques. (I)
minimizes the hydrolysis of transglycosylation products and consequently
improves transglycosylation yields, in comparison with wild-type
glycosidase.

EXAMPLE - The ***gene*** (wild type, lac S) encoding the thermophilic, retaining, exo-beta-glycosidase, was isolated from Sulfolobus solfataricus MT-4 strain (SSbetaG). The wild type lac S, was then amplified by PCR using primers having sequences such as 5'-CCATGGGACACCACCACCACCACCACTCATTAC-3' and 3'-CPCGAGTTAGTGCCTTTATGGCTTTACTGGAGGTAC-5'. The PCR product was cloned into pCR2.1. Mutations were introduced into the lac S ***gene*** coding ***sequence*** (in pCR2.1) according to Stratagene QuickChange mutagenesis system. Mutated coding ***sequence*** were cloned into the Ncol/Xhol sites of expression vector pET-24-d(+), and transformed into Escherichia coli BL21 (DE3). Putative transformants were identified by colony PCR using SSbetaG coding ***sequence*** primers. Selected clones were grown in LB medium containing kanamycin (50 micrograms/ml) at 37degreesC to an optical density of 0.6 at 600 nm, and the target protein was induced by addition of 0.1 M isopropyl-beta-D-thiogalactopyranoside (IPTG). Then, the cells were obtained, lysed, and the ***modified*** glycosidase were purified using nickel-chelation chromatography. The 28 mg/L (95% purity) of E387Y SsbetaG ***mutant*** enzyme was obtained.(50 pages)

L8 ANSWER 4 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN ACCESSION NUMBER: 2004-07676 BIOTECHDS <<LOGINID::20060918>>

TITLE: Novel mutant Chrysosporium strain comprising nucleic acid sequence encoding polypeptide of interest such as proteases and lipases, the nucleic acid sequence being operably linked to expression-regulating region;

involving vector-mediated gene transfer and expression in host cell

AUTHOR: EMALFARB M A; BURLINGAME R P; OLSON P T; SINITSYN A P; PARRICHE M; BOUSSON J C; PYNNONEN C M; PUNT P J; VAN ZEIJL C

PATENT ASSIGNEE: EMALFARB M A; BURLINGAME R P; OLSON P T; SINITSYN A P; PARRICHE M; BOUSSON J C; PYNNONEN C M; PUNT P J; VAN ZEIJL C

PATENT INFO: US 2004002136 1 Jan 2004
APPLICATION INFO: US 2003-394568 21 Mar 2003
PRIORITY INFO: US 2003-394568 21 Mar 2003; WO 1998-6496 6 Oct 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-061663 [06]
AN 2004-07676 BIOTECHDS <<LOGINID::20060918>>
AB DERWENT ABSTRACT:

NOVELTY - A ***mutant*** Chrysosporium strain (I) comprising a nucleic acid ***sequence*** encoding a polypeptide of interest, the being operably linked to an

expression-regulating region and optionally a secretion signal **sequence***, the ***mutant*** strain expressing the polypeptide of interest at a higher level than the corresponding non- ***mutant** strain under the same conditions, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a nucleic acid construct (II) comprising a nucleic acid expression-regulatory region derived from C.lucknowense, operably linked to a polypeptide-encoding nucleic acid ***sequence*** (2) a recombinant microorganism (III) containing (II), and capable of expressing the polypeptide encoded by the coding nucleic acid ***sequence***; (3) producing (M1) (I), involves stably introducing a nucleic acid ***sequence*** encoding a heterologous or homologous polypeptide into a Chrysosporium strain, the nucleic acid ***sequence*** being operably linked to an expression regulating region; (4) a protein (P1) corresponding to a Chrysosporium ***glycosyl*** ***hydrolase*** family 7, exhibiting at least 75% amino acid identity as determined by the BLAST algorithm with a fully defined ***sequence*** of 465 amino acids (S1) as given in specification, or its part having at least 20 contiguous amino acids which are identical to the corresponding part of the amino acid ***sequence*** 1-246 or 394-526 of (S1); (5) a protein (P2) corresponding to a Chrysosporium ***glycosyl*** ***hydrolase*** family 10, exhibiting at least 70% or 65% amino acid identity as determined by the BLAST algorithm with a fully defined ***sequence*** of 465 amino acids (S2), or its part having at least 20 contiguous amino acids which are identical to the corresponding part of the amino acid ***sequence*** 1-383 of (S2); (6) a protein corresponding to a Chrysosporium ***glycosyl*** ***hydrolase*** family 10 and comprising a cellulose-binding domain chosen from (a) domains having at least 75% amino acid identity with the amino acid ***sequence** 22-53, and (b) domains having at least 20 contiguous amino acids identical to a part of amino acid ***sequence*** 22-53 of (S2), (7) a fungal ***glycosyl*** ***hydrolase*** of family 10, comprising a cellulose-binding domain, not derived from Fusarium oxysporum; (8) a protein (P3) corresponding to a Chrysosporium ***glycosyl*** ***hydrolase*** family 12, (9) a protein (P4) corresponding to a Chrysosporium glyceraldchyde phosphate dehydrogenase; (10) a protein (P5) corresponding to a Chrysosporium ***glycosyl*** ***hydrolase*** family 45; (11) a protein (P6) corresponding to a Chrysosporium ***glycosyl*** ***hydrolase*** family 6; (11) a nucleic acid ***sequence*** (IV) encoding any one of the proteins from (P1)-(P6); (12) a nucleic acid ***sequence*** comprising at least 70% of the nucleotides contained in the 5'-noncoding region of a nucleic acid ***sequence*** (NA) chosen from tccaaaccctaaagctgatatcac, cetggatagettetgtecatte, ttattttttecaggtacecagcatgee and a fully defined ***sequence*** of 1570 nucleotides or 74 nucleotides as given in specification; (13) a nucleic acid construct (V) comprising a nucleic acid expression-regulatory region derived from Chrysosporium, contained in the 5'-noncoding region of (NA), operationally linked to a nucleic acid ***sequence*** encoding a polypeptide of interest; (14) a recombinant microbial strain (VI) containing (V), and capable of expressing the polypeptide encoded by the coding nucleic acid ***sequence***; (15) a recombinant microbial strain (VII) containing (IV), and capable of expressing the polypeptide encoded by the coding nucleic acid ***sequence***; and (16) an oligonucleotide probe (VIII) comprising at least 15 contiguous nucleotides of the (NA), or its complement. BIOTECHNOLOGY - Preferred ***Mutant*** : (1) is obtained by recombinant methods which involves stable introduction of at least one heterologous nucleic acid ***sequence*** chosen from heterologous polypeptide-encoding nucleic acid sequences, heterologous signal sequences and heterologous expression-regulating sequences. The polypeptide of interest is a heterologous polypeptide of plant, animal (including human), insect, algal, bacterial, archaebacterial or fungal origin. The polypeptide of interest is a homologous polypeptide which is expressed at a higher level than in the corresponding non- ***mutant*** strain under the same conditions. The polypeptide of interest is chosen from ***carbohydrate*** - ***degrading*** enzymes, proteases, lipases, esterases, other hydrolases, oxidoreductases and transferases. The polypeptide of interest is chosen from fungal enzymes allowing

(over)production of primary metabolites, including organic acids, and secondary metabolites, including antibiotics. The polypeptide of interest is inactivated at a pH below 6. The polypeptide of interest exhibits optimal activity and/or stability at a pH above 6, and/or has more than 70% of its activity and/or stability at a pH above 6. (I) comprises a heterologous signal ***sequence*** . (I) comprises a fungal signal ***sequence*** is a signal ***sequence*** of a cellulase, beta-galactosidase, xylanase, pectinase. esterase, protease, amylase, polygalacturonase or hydrophobin. (I) further comprises a selectable marker. The selectable marker confers resistance to a drug or relieves a nutritional defect. (I) comprises a heterologous/fungal expression-regulating region. The expression-regulating region comprises an inducible promoter or high expression promoter. (1) is obtained by mutagenesis steps, the steps including at least one step chosen from UV irradiation and chemical mutagenesis. The mutagenesis steps comprise a first UV irradiation step, a N-methyl-N'-nitro-N-nitrosoguanidine treatment step, and a second UV irradiation step. (1) is derived from Chrysosporium lucknowense. The ***mutant*** is derived from a C.lucknowense ***mutant*** strain chosen from C.lucknowense strain C1 (VKM F-3500 D), UV13-6 (VKM F-3632 D), NG7C-19 (VKM F-3633 D), and UV18-25 (VKM F-3631 D). When a Trichoderma reesei strain and the C.lucknowense strain are cultured under equivalent optimal conditions, when the Trichoderma culture strains a viscosity of 200-600 cP, the Chrysosporium strain exhibits a biomass of less than half that of the Trichoderma. The strain producing at least the amount of cellulase in moles per liter as produced by any of the C.lucknowense ***mutant*** strains C1 (VKM F-3500 D), UV13-6 (VKM F-3632 D), NG7C-19 (VKM F-3633 D), and UV18-25 (VKM F-3631 D). The strain produces less protease than produced by the C.lucknowense strain C1 (VKM F-3500 D). The strain produces less than half the amount of protease produced by the C1 strain. Preferred Construct: In (II), the expression-regulatory region is derived from a C.lucknowense strain chosen from C1 (VKM F-3500 D) and UV18-25 (VKM F-3631 D). The expression-regulatory region comprises a promoter ***sequence** associated with cellulase expression, xylanase expression, or gpdA expression. Preferred Microorganism: (III) is a fungal strain. Preferred Method: In (M1), the nucleic acid is introduced by the protoplast transformation method. Preferred Probe: (VIII) is 20-50 nucleotides in length and is labeled with a detectable label.

USE - (I) is useful for producing a polypeptide of interest by culturing (1) under conditions permitting expression of the protein or polypeptide, and recovering the subsequently produced polypeptide of interest. The conditions further permit secretion of the protein or polypeptide of interest. (III) is useful for producing a polypeptide of interest by culturing (III) under conditions permitting expression of the protein or polypeptide and recovering the subsequently produced polypeptide of interest. The protein or polypeptide is expressed as a precursor protein, and further involves cleavage of the precursor into the polypeptide or precursor of interest. The cleavage step is cleavage with an enzyme chosen from Kex-2 like proteases, basic amino acid paired proteases, or Kex-2. The cultivation occurs at pH in the range 6-9, and/or at a temperature between 25-43degreesC. (P1), (P3), (P5), or (P6) is useful for hydrolyzing beta-glucosidic bonds by contacting a beta-glucoside with (P1), (P3), (P5), or (P6). (P2) is useful for hydrolyzing beta-xylosidic bonds by contacting a beta-xyloside with (P2). (V), (VI) or (VII) is useful for producing a polypeptide (claimed).

EXAMPLE - C1 strains NG7C-19 and UV18-25 of Chrysosporium lucknowense were transformed with plasmid pUT7201. The vector had a fungal expression cassette, which had Aspergillus nidulans glyceraldehyde-3-phosphate dehydrogenase (gpdA) promoter2, a synthetic Trichoderma reesei cellobiohydrolasel (cbh1) signal ***sequence*** (1.3), Streptoalloteichus hindustanus phleomycin-resistance ***gene*** Sh ble4, A.nidulans tryptophan-synthase (trpC) terminator5. The vector also carried the beta-lactamase ***gene*** (bla) and Escherichia coli replication origin from plasmid pUC186. C1 protoplasts were transformed according to Durand et al. Primary transformants were toothpicked to glutamine synthetase (GS)+phleomycin plates and grown for 5 days at 32degreesC for resistance verification. Each validated resistant clone was subcloned onto GS plates. Two-subclones per transformant were used to inoculate PDA plates in order to get spores for liquid culture

initiation. The liquid cultures in ICI were grown 5 days at 27degreesC. Then, the cultures were centrifuged and 500 microl of supernatant were collected. From the samples, the proteins were precipitated with TCA and resuspended in Western Sample Buffer to 4 mg/ml of total proteins. Ten microl were loaded on a 12% acrylamide/sodium dodecyl sulfate (SDS) gel and run. Western blotting was conducted using rabbit ant-Sh ble antiserum as primary antibody. The results showed that the heterologous transcription/translation signals from pUT720 were functional in Chrysosporium, and the secretion of human lysozyme was also confirmed. (70 pages)

L8 ANSWER 5 OF 6 FSTA COPYRIGHT 2006 IFIS on STN

ACCESSION NUMBER: 2002:B1506 FSTA <<LOGINID::20060918>>

Cloning and structural analysis of bglM gene coding TITLE

for the fungal cell wall-lytic .beta.-1,3-glucanhydrolase BgIM of Bacillus circulans IAM1165.

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AB Bacillus circulans IAM1165 produces multiple isoforms of .beta.-1,3-glucan hydrolases. Of these the 42 kDa endo-1,3(4)-.beta.-glucanase (EC 3.2.1.6) BglM is the most active toward Aspergillus oryzae cell walls. A

gene coding for a BglM precursor, consisting of 411 amino acid residues was cloned and sequenced. The native and recombinant enzyme (expressed in Escherichia coli) and a recombinant deletion ***mutant*** were characterized. The 27 N-terminal amino acid ***sequence*** of the precursor was shown to be a signal peptide. The 141 C-terminal amino acid ***sequence*** contained family 13 ***carbohydrate*** -binding which bound to pachyman, lichenan and A. oryzae cell walls. The central domain showed a bacterial .beta.-1,3-glucan hydrolase motif belonging to ***glycosyl*** ***hydrolase*** family 14. By removal of the C-terminal domain B. circulans IAM1165 ***processed*** mature BglM to

several 27 kDa fragments that hydrolysed a soluble .beta.-1,3-glucan. L8 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

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TITLE: Analysis of the chromosome sequence of the legume

symbiont Sinorhizobium meliloti strain 1021

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AB Sinorhizobium meliloti is an .alpha.-proteobacterium that forms agronomically important N2-fixing root nodules in legumes. We report here

the complete sequence of the largest constituent of its genome, a 62.7% GC-rich 3654,135-bp circular chromosome. Annotation allowed assignment of a function to 59% of the 3341 predicted protein-coding ORFs, the rest exhibiting partial, weak, or no similarity with any known sequence. Unexpectedly, the level of reiteration within this replicon is low, with only two genes duplicated with more than 90% nucleotide sequence identity, transposon elements accounting for 2.2% of the sequence, and a few hundred short repeated palindromic motifs (RIME1, RIME2, and C) widespread over the chromosome. Three regions with a significantly lower GC content are most likely of external origin. Detailed annotation revealed that this replicon contains all housekeeping genes except two essential genes that are located on pSymB. Amino acid/peptide transport and degrdn. and sugar metab. appear as two major features of the S. meliloti chromosome. The presence in this replicon of a large no. of nucleotide cyclases with a peculiar structure, as well as of genes homologous to virulence determinants of animal and plant pathogens, opens perspectives in the study of this bacterium both as a free-living soil microorganism and as a plant symbiont.

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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L2 QUE (GLYCOSYL (W) HYDROLASE)

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- L4 1563 S (GENE OR SEQUENCE OR POLYNUCLEOTIDE)(S)L3
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